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DNA Vaccination for HIV-1 and SIV

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DNA vaccine · Immunization, genetic · HIV-1 · SIV

Abstract

Control of the worldwide AIDS epidemic will only be achieved with a safe and effective prophylactic HIV-1 vaccine. DNA vaccination has recently emerged as a promising vaccine modality that can elicit both humoral and cellular immune responses. HIV-1- and SIV-specific immune responses have been elicited by DNA vaccines in both mice and nonhuman primates. However, these immune responses have not been capable of protecting nonhuman primates against pathogenic AIDS virus challenges. A number of approaches are therefore being investigated to augment DNA vaccine-elicited immune responses.

Introduction

Over 30 million individuals are infected with HIV-1 worldwide. Although the development of highly active antiretroviral therapy represents a major advance for HIV-infected individuals in the western industrialized world, over 90% of HIV-infected individuals worldwide will never benefit from these therapies. It is generally agreed that a safe and effective vaccine will be the only way to control the global AIDS epidemic [1, 2].

Over the past several years there has been a dramatic increase in our understanding of AIDS pathogenesis and the immune responses to HIV-1 infection in humans and SIV infection in rhesus macaques. A wealth of data has established that a vigorous cellular immune response is required for the control of HIV-1 and SIV replication. The early appearance of a robust cytotoxic T lymphocyte (CTL) response was shown to occur coincident with the decline in primary viremia during HIV-1 infection in humans [3–5]. In addition, virus-specific CTL appear to play a critical role in controlling chronic HIV infection [6]. Moreover, a significant inverse correlation was demonstrated between the frequency of HIV-specific CTL and viral load, supporting the significance of the role of CTL in controlling HIV-1 infection [7]. Finally, a vigorous virus-specific helper T cell response has also been shown to correlate with control of viremia [8].

During SIV infection in rhesus macaques, the CTL response appears critical for controlling viral replication. During primary SIV infection, a dramatic rise in circulating and lymph node SIV-specific CTL was found to occur coincident with the control of primary viremia [9–11]. In addition, two groups recently demonstrated that depletion of CD8+ lymphocytes during SIV infection resulted in a dramatic increase in viremia, directly demonstrating the importance of CTL in vivo [12, 13].

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These data suggest that an effective HIV-1 vaccine must elicit potent cellular immune responses. HIV-1 vaccine development strategies have been recently reviewed [2]. Current strategies include immunization with live attenuated viruses, whole killed viruses, protein subunits, recombinant live vectors, and plasmid DNA. Live attenuated viruses have been shown to generate neutralizing antibody and CTL responses, but the safety of this approach has been questioned. Whole killed viruses and protein subunits are limited by their inability to elicit CTL responses. In contrast, recombinant live vectors and plasmid DNA vaccines can generate cellular immune responses with little or no infectious risks. DNA vaccines offer the additional advantage of being simple and inexpensive to construct, easy to produce in large quantities, and stable for long periods of time. If DNA vaccination proves to be efficacious, production and delivery to individuals in developing nations may be more economically and logistically feasible than with other types of vaccines.

DNA Vaccines for HIV-1 and SIV

DNA vaccination involves the administration of purified plasmid DNA encoding an antigen. Plasmid DNA is typically injected into skeletal muscle or inoculated as plasmid-coated beads by gene gun into the epidermis. The protein is expressed in transfected mammalian cells, including macrophages and dendritic cells, enters into both the MHC class I and class II processing pathways, and elicits strong and persistent humoral and cellular immune responses [14, 15, reviewed in 16–18]. The potential clinical utility of this vaccine modality was demonstrated by Ulmer et al. [19]. In this report, intramuscular injection of a purified plasmid encoding influenza A nucleoprotein elicited potent antigen-specific CTL responses in addition to antibody responses. These immune responses were sufficient to protect mice against challenge with a heterologous strain of influenza A virus.

The potential utility of plasmid DNA as a component of an HIV-1 vaccine has been an area of active investigation. DNA vaccination with plasmids encoding HIV-1 *env* was first shown to elicit Env-specific humoral and cellular immune responses in both mice [20–22] and macaques [23]. A DNA vaccine expressing a soluble form of HIV-1 gp120 also was shown to generate specific antibodies, CD8+ antigen-specific CTL responses, and T_H1-like CD4+ helper T cell-proliferative responses in mice and macaques [24–27]. The immune responses were dose-dependent, boostable and long-lived (>6 months) [25, 28]. SIV-specific CTL responses were also elicited in macaques by DNA vaccination with a plasmid containing SIV *env* and *gag* [29].

Several viral challenge studies have been performed in nonhuman primates to evaluate the protective capacity of the immune responses elicited by DNA vaccines. Complete protection against high-dose challenge with the SF2 strain of HIV-1 was shown following DNA vaccination of chimpanzees with plasmids containing HIV-1 *env* and *gag/pol* [30]. However, the significance of this study remains uncertain, since HIV-1 SF2 replication in chimpanzees occurs at very low levels and is nonpathogenic [31]. In rhesus macaques, six immunizations with DNA encoding SIV *env* genes failed to protect against intravenous challenge with the virulent SIVmac251 isolate [32]. This study did show, however, that viral load was reduced and pathogenicity was attenuated in the vaccinated animals. A second study showed that DNA vaccination of pigtail macaques led to a reduction in viral loads following intrarectal challenge with SIVmne, a viral isolate of intermediate pathogenicity [33]. A study from our group recently demonstrated that SIV *gag* DNA vaccination of rhesus macaques led to the development of secondary CTL responses and reduced viral loads following intravenous challenge with the highly pathogenic SIVmn E660 virus [Egan and Letvin, unpubl. data]. Thus, DNA vaccination with HIV-1 or SIV antigens elicits humoral and cellular immune responses in nonhuman primates. While these results appear promising, DNA vaccine-elicited immune responses have not yet protected primates against a pathogenic viral challenge.

DNA vaccines encoding HIV-1 antigens are also being investigated as potential therapies to augment specific immune responses in HIV-infected patients. Several preliminary studies have shown that DNA vaccines can enhance proliferative T cell or CTL activity [34–36]. However, clinical efficacy of such strategies has not yet been demonstrated.

Strategies to Augment DNA Vaccine-Elicited Immune Responses

The failure of DNA vaccines to generate immune responses capable of protecting nonhuman primates against pathogenic viral challenges has led a number of investigators to develop strategies to augment these immune responses. Two such strategies deserve particular mention: prime-boost approaches and the coadministration of immunomodulator molecules.

Prime-Boost Approaches

One possible approach to augment immune responses elicited by DNA vaccines involves the combination of multiple vaccination modalities. Such multimodal vaccine approaches have largely focused on boosting DNA-primed immune responses with recombinant proteins or recombinant live vectors. DNA priming followed by Env IIIB protein boosting increased antibody responses and successfully protected rhesus macaques against challenge with the nonpathogenic SHIV-IIIB virus [37]. A similar study showed that protein boosting increased DNA-primed antibody responses in cynomolgous macaques, but the immunization regimen described in this study did not result in protection against a nonpathogenic SHIV-Lai challenge [38]. A study in rabbits primed with Env-expressing plasmids showed that Env IIIB protein boosting increased the vaccine-elicited antibody titers and neutralizing activity [39]. Protein boosting thus appears to augment the neutralizing antibody responses to T cell line-adapted, nonpathogenic viruses but has not been able to generate broadly reactive neutralizing antibody responses or provide protection against pathogenic viruses.

A second multimodal vaccine approach is boosting DNA-primed immune responses with recombinant live vectors. DNA priming followed by boosting with recombinant modified vaccinia Ankara (MVA), a pathologically attenuated pox virus, led to high frequency CTL responses and protection of mice against a *Plasmodium berghei* sporozoite challenge [40, 41]. A similar strategy of priming with an SIV Gag epitope DNA vaccine and boosting with recombinant MVA induced potent Gag-specific CTL responses in rhesus macaques [42]. The CTL responses elicited by this approach were more potent that those elicited by DNA alone or recombinant MVA alone. No protection was observed, however, against a pathogenic intrarectal SIVmac251 challenge in at least 2 of the 3 vaccinated animals in this study. Vaccination with DNA plus recombinant vaccinia also did not more effectively control a viral challenge than immunization with DNA alone [43]. Several recent studies in nonhuman primates have evaluated a regimen involving priming with DNA and boosting with recombinant fowlpox viruses. Vaccination of macaques with DNA plus recombinant fowlpox virus led to augmented cellular immune responses and decreased pathogenicity following challenge with a nonpathogenic HIV-1 [44] or a nonpathogenic SHIV-IIIB [45]. In this latter study, the macaques that controlled two SHIV-IIIB challenges also were shown to control a pathogenic SHIV-89.6P challenge. However, it is possible that this protection was mediated in part by the prior SHIV-IIIB exposures.

Recombinant vectors thus appear to have the ability to augment the cellular immune responses primed by DNA vaccines. In certain cases boosting with a recombinant vector has resulted in enhanced protection against nonpathogenic viral challenges. The degree to which boosting with live vectors will augment the ability of nonhuman primates to control pathogenic AIDS virus challenges remains to be determined.

Immunomodulator Molecules

Another strategy for augmenting DNA vaccine-elicited immune responses involves the coadministration of plasmids encoding immunomodulator molecules such as cytokines, chemokines, costimulatory molecules and adhesion molecules. The possibility of rationally designing vaccines or manipulating immune responses has aroused considerable recent interest in this approach.

The utility of plasmid cytokines to modulate DNA vaccine-elicited immune responses was first shown by the demonstration that coinoculation of plasmid GM-CSF enhanced, but plasmid IFN-γ suppressed, the antibody and proliferative T cell responses elicited by a rabies virus-specific DNA vaccine [46]. A large number of studies have since investigated the ability of plasmid cytokines to augment immune responses to DNA vaccines specific for a broad array of antigens including HBV, HCV, HIV-1, influenza, *Plasmodium* and *Leishmania* in small animals. Augmentation of DNA vaccine-elicited HIV-specific cellular immune responses in mice has been reported by the coadministration of plasmid GM-CSF [47, 48], IL-2 [48, 49], IL-12 [47, 48, 50] and IL-15 [51, 52]. Other reports have described the augmentation of DNA vaccine-elicited HIV-1-specific immune responses in mice using plasmids expressing the costimulatory molecule B7-2 [53–55], the adhesion molecules ICAM-1 and LFA-3 [56], and the chemokines RANTES, MIP-1α and MCP-1 [57–59].

A report by our group shows that a plasmid encoding the fusion protein IL-2/Ig, a protein that has IL-2 activity and a longer half-life in vivo, is more effective than plasmid IL-2 in augmenting DNA vaccine-elicited HIV-1 Env-specific antibody and CTL responses in mice [60]. This study also suggests that simultaneous administration of plasmid cytokines with DNA vaccines may not optimally harness this technology, since the highest immune responses were seen when plasmid IL-2/Ig was administered 2 days after the vaccine. We have also demonstrated that plasmid IL-2/Ig administration can substantially aug-

ment antibody and CTL responses elicited by HIV-1 and SIV DNA vaccines in rhesus macaques [Barouch and Letvin, unpubl. results]. Thus, a number of strategies exist for augmenting DNA vaccine-elicited immune responses in both mice as well as nonhuman primates. Whether the degree of augmentation seen to date with these strategies is sufficient to affect clinical parameters following a pathogenic viral challenge is not yet known.

Other Strategies

A number of other strategies for augmenting DNA vaccine-elicited immune responses also are under active investigation. These approaches include changing the vaccine backbone to increase antigen expression by optimizing the usage of mammalian codons in the foreign genes inserted into the DNA vaccines and by increasing the number of CpG sequences in the plasmids to provide the greatest adjuvant effect. Other strategies include attempts to improve the delivery of the vaccine through the use of needle-free injection devices, electroporation to improve the frequency of in vivo transfection, and the development of specific methods to target dendritic cells. Formulations of DNA vaccines with novel chemical and lipid adjuvants are also being assessed.

It has been difficult to generalize from the HIV-1 and SIV vaccine studies in nonhuman primates done to date. Not only have a diversity of immunization regimens been employed, but a variety of markedly different challenge viruses have been used. In the various studies that have been reported, the plasmid DNA constructs employed have differed in their construction and in the methods by which they have been delivered as immunogens. It is clear now that the immunogenicity of plasmid DNA vaccines in higher primates is dependent on the promoters utilized for antigen expression, whether or not the plasmids have been codon-optimized for maximal expression in mammalian cells, the quantity and purity of the DNA used in each inoculation, the route of administration, and the precise formulation of the vaccine. It is also clear that immune protection in nonhuman primates is much more readily achieved against nonpathogenic viruses than against highly virulent pathogenic viruses.

Conclusions

Although the correlates of immunity for vaccine protection against HIV-1 infection have not been definitively established, accumulating evidence over the past several years suggests that candidate AIDS vaccines should generate potent CTL responses as well as neutralizing antibody responses. Plasmid DNA vaccines expressing HIV-1 or SIV antigens are promising in their ability to elicit both cellular and humoral immune responses in mice and nonhuman primates without the infectious risks associated with immunization using attenuated viruses or certain live recombinant vectors. However, immune responses elicited by DNA vaccines alone are unlikely to be of sufficient magnitude to achieve protective immunity against pathogenic AIDS virus challenges. Significant attention, therefore, has focused on developing methods of augmenting DNA vaccine-elicited immune responses, including boosting DNA-primed responses with recombinant proteins or recombinant live vectors and coimmunizing with immunomodulatory molecules. Preliminary data suggest that these strategies can result in significantly augmented HIV-1- and SIV-specific immune responses. However, none of these approaches has yet been shown to protect nonhuman primates against a pathogenic AIDS virus challenge.

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